UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

AUG 07 2003

**MEMORANDUM**

**SUBJECT:** Science Review in Support of the Registration of Zonix™ Biofungicide (EPA File Symbol No. 072431-R), Containing 8.5% Rhamnolipid Biosurfactant [Decanoic acid, 3-[[6-deoxy-2-O-(6-deoxy-α-L-anno-pyranosyl)-α-L-mannopyranosyl]oxy]-1-(carboxymethyl)octyl ester, mixture with 1-carboxymethyl)octyl-3-[(6-deoxy-α-L-mannopyranosyl)oxy]decanoate]; (Chemical No. 105120)] As Its Active Ingredient. Review of Product Chemistry Data/Information and a Honey Bee Contact Toxicity Study; and Addenda to a Petition for an Exemption From the Requirement of Food Tolerances (PP#1F06288). DP Barcodes D289391 & 289392; Case Nos. 070286 & 294050; Submission Nos. S632803 & 632806; MRID 457852-01.

**FROM:** Russell S. Jones, Ph.D., Biologist
Biochemical Pesticides Branch
Biopesticides & Pollution Prevention Division (7511C)

**TO:** Denise Greenway, Regulatory Action Leader
Biochemical Pesticides Branch
Biopesticides & Pollution Prevention Division (7511C)

**ACTION REQUESTED**

In response to a request for additional information (see Memorandum from R. S. Jones to D. Greenway, dated 11/21/2003; and letter from A. P. Roberts to D. Greenway, dated 12/23/2002), the registrant has submitted a revised Confidential Statement of Formula (CSF; dated 03/06/2003) and other product chemistry information; and supplemental reference citations in support of waivers for toxicity and non-target organisms. In addition, a honey bee contact toxicity study was submitted for review.

**THE FOLLOWING PAGES CONTAIN CONFIDENTIAL BUSINESS INFORMATION (CBI)**

**Manufacturing process information may be entitled to confidential treatment**

## CONCLUSIONS AND RECOMMENDATIONS

1a. The revised CSF (dated 3/6/2003 is acceptable; no additional data are required. The revised CSF adequately describes the components of the active ingredient and the impurities resulting from [redacted] during manufacturing (a copy of the revised CSF is attached to this review).

1b. The certified limits for the components of the active ingredient are acceptable. Although the certified limits for the components of the active ingredient are wider than those recommended by 40 CFR 158.175, the rationale provided by the registrant to explain the proposed certified limits is acceptable.

The two components of the active ingredient [designated R1 and R2 in the end-use product (see revised CSF)] are produced concurrently during the [redacted] portion of the manufacturing process. According to the registrant, the relative amounts of R1 and R2 produced [redacted] are a function of the genetics of the bacterium and cannot be practically controlled to narrower certified limits. Furthermore, the efficacy of the end-use product depends primarily on the total percentage of R1 and R2 in the product, not the percentages of the individual components.

1c. The description of the manufacturing process is upgraded to acceptable. The registrant has provided the suppliers of all beginning materials and has clarified a discrepancy in the original submission (MRID 453767-01) regarding the [redacted] needed during the production of the active ingredient. [redacted].

1d. The discussion of the formation of impurities is upgraded to acceptable. The registrant has adequately described the function of [redacted] used in the manufacturing process and how it is removed from the product.

[redacted]

1e. The physical/chemical properties are upgraded to acceptable. The methods whereby these properties were also submitted (photocopy attached to review). The following physical chemical properties were submitted:

| Physical/Chemical Property | TGAI | EP |
|---|---|---|
| Color | Transparent with a light to dark amber tint | Not required for EP (NR) |
| Physical State | Not required for TGAI (NR) | Liquid |
| Odor | Mild, sweet, soapy | Not required for EP |

| Physical/Chemical Property | TGAI | EP |
|---|---|---|
| Stability | Stable at normal and elevated temperatures | NR |
| Oxidation/Reduction | NR | Does not contain oxidizing or reducing agents |
| Flammability | NR | Does not contain combustible liquids |
| Explodability | NR | Not explosive |
| Storage Stability | NR | Stable for one year when stored in the original container per label instructions |
| Miscibility | NR | Not Applicable (NA) |
| Corrosion Characteristics | NR | Not corrosive |
| Dielectric Breakdown Voltage | NA | NA |
| Viscosity | NR | 1.0 mPas |
| Melting Point | NA | NA |
| Boiling Point | 99.0 °C | NR |
| Bulk Density/Specific gravity | 8.514 | 8.345 |
| Dissociation Constant | NA | NA |
| Partition Coefficient | NA | NA |
| Water Solubility | Soluble in water | NR |
| Vapor Pressure | 31 K pa | NR |

2a Hypersensitivity data requirements are upgraded to acceptable. Relevant portions of the patent applications [U. S. Patents 5,455,232 (Pilchek) and 4.902,512 (Ishigami)] were submitted as Attachments 3 to the letter from A. P. Roberts to D. Greenway (dated 12/23/2002). The aforementioned data/information demonstrate that the active ingredient is not a sensitizer.

2b. A specific list of products that are currently in use as emulsifiers, dispersants, wetting agents, and agricultural adjuvants, as well as the dates of the introduction of these products into the market place was submitted by the registrant and is acceptable (see Table in Attachment 4 to the letter from A. P. Roberts to D. Greenway (dated 12/23/2002). Additionally, a technical journal article (*Lang & Wullbrandt, 1999. Rhamnose lipids - biosynthesis, microbial production, and application potential. Appl. Microbiol. Biotechnol. 51: 22-32*) detailing how rhamnolipids have potential applications in oil pollution remediation and fungal phytopathogens, as well as its current use as a source of L-rhamnose, a sugar used in the flavor industry.

The first listed commercial introduction of rhamnolipid was in 1989 as a crude oil tank cleaner and the most recent commercial introduction was in 2003 as a cleaning product. In all, Attachment 4 lists ten categories of products (including shampoos, oil dispersants, fuel additive, agricultural adjuvants, skin creams/cosmetics, soil remediation products, surfactants, and degreasers) introduced to the marketplace between 1989 and 2003

2c. Waivers for genotoxicity and 90-day oral, dermal, and inhalation toxicity studies, teratogenicity, and immune response are upgraded to acceptable. The registrant has submitted references pertaining to food uses of rhamnose and the fatty acid, hydroxydecanoic acid (see Attachments 5 and 6 to the letter from A. P. Roberts to D. Greenway (dated 12/23/2002) as requested in the Memorandum from R. S. Jones to D. Greenway, dated 11/21/2002). There has been regular exposure of humans to rhamnolipids via diverse agricultural, cosmetic, and industrial uses for a minimum of approximately 15 years (see Conclusion 2b above), as well as widespread oral exposure to the components of rhamnolipid (rhamnose and hydroxydecanoic acid) via their use as food additives (see below). There have been no reported adverse effects to rhamnolipid, rhamnose, or hydroxydecanoic acid via any route of exposure.

Rhamnose (a component of the rhamnolipid active ingredient) is also a component of Acacia (gum arabic) which is listed as Generally Recognized As Safe (GRAS) by Food and Drug Administration [FDA (see 21 CFR 184.1330)]; acacia is a common food additive that has been evaluated as safe by the World Health Organization (WHO), the Food and Agriculture Organization (FAO), and FDA. See the following references in Attachment 5:

Rulis, AM, 2001. USFDA. Agency response letter GRAS notice no. GRN 000058. Letter to Mr. John Lemker, Bell, Boyd, and Lloyd, LLC.

Scheidin, S. 2001. J. Amer. Pharm. Assoc. 41(5): September/October 2001.

WHO, Food Additive Series 26. Gum Arabic, 686. www.inchem.org/documents/jefca/jecmono/v26je07.htm

FAO, 1997. Gum Arabic. Joint Expert Committee on Food Additives (JEFCA) 44th Meeting.

Hydroxydecanoic acid (a component of the rhamnolipid active ingredient) is a fatty acid component of honey bee royal jelly, a commonly used dietary supplement. See the following references in Attachment 6:

Enterex. Super Royal Jelly. Description of food supplement contents including hydroxydecanoic acid. www.enterex.bc.ca/products/superroyaljelly.htm

JEFCA 1997. Gamma-Decalactone. Joint FAO/WHO Expert Committee on Food Additives. Report: TRS 884-JEFCA 49/42. Monograph 40-JEFCA 49/231.

Krell, R. 1996. Value-added products from beekeeping. Chapter 6: Royal Jelly. FAO Agricultural Services Bulletin No. 124. www.fao.org/docrep/w0076e16.htm

Royal Society of Chemistry. 2001. The chemistry of bees: Royal jelly. www.chemsoc.org/exemplarchem/entries/2001/loveridge/index-pages6.htm

Shigeru et al. 1998. Chemical Ecology in the Japanese honeybee, *Apis cerana japonica rad.*(acj.). International Society of Chemical Ecology Annual Meeting, 1998. Abstract: O-16.

Simon, U. 1998. Regulation of reproductive dominance hierarchies in Apis mellifera capensis workers. Chapters 2 & 3. Dissertation, Martin Luther Univ., Halle, Wittenberg, Germany.

2d. An acceptable contact honeybee toxicity study was submitted. The product (containing 8.5% rhamnolipid) is slightly toxic to honeybees:

4-hr $LD_{50}$ > 25 µg/bee; 24-hr $LD_{50}$ = 20 µg/bee; and 48-hr $LD_{50}$ = 19 µg/bee

**NOTE TO RAL:** No bee precautionary statements are required on the label. However, if registrations are requested for new rhamnolipid-containing products containing >8.5% rhamnolipid, new acute contact honeybee studies using those products will be required as a condition of the registration of those new products.

## STUDY SUMMARY

Non-Target Organisms

The registrant submitted a contact honeybee study in MRID 457852-01. Adult honeybees (*Apis mellifera*) obtained from a commercial apiary were anesthetized with $CO_2$ and each bee was treated with a 1 µL drop of end use product (containing 8.5% rhamnolipid) to the dorsal side of the thorax of each bee. In a definitive test, bees were treated with test substance at concentrations of 1.9, 3.2, 5.4, 9.0, 15 and 25 µg a.i./bee. The untreated control bees were treated with water only and a positive control (dimethoate) was also applied to bees. Each treatment was replicated in three cages for a total of 30 bees per treatment. The bees were then incubated for 48 hours following treatment and observations of mortality and toxicity were conducted at 4, 14 and 48 hours after post-treatment. Mortality was 0, 13, 13, 23, 27 and 77% for the bees exposed to treatments of 1.9, 3.2, 5.4, 9.0, 15 and 25 µg a.i./bee, respectively. No mortality was observed in the untreated control or the carrier control. In the positive control, mortality ranged from 0 to

100% in the individual dimethoate treatments (0.020, 0.20 and 0.40 µg dimethoate). The following $LD_{50}$s were calculated:

4-hr $LD_{50}$ > 25 µg/bee;
24-hr $LD_{50}$ = 20 µg/bee; and
48-hr $LD_{50}$ = 19 µg/bee

Based on the data, the product is slightly toxic to bees.

No data were submitted for the TGAI. The product label states that the product is to be applied at a concentration of 85 ppm. Since the product contains 8.5% a..i. the applied rate is calculated to be 7.225 mg a.i./L (85 mg/L x 0.085). If applied according to label directions at 100 gallons (378.5 L)/acre, the active ingredient is applied at a rate of approximately **2.7 g a.i./acre** (378.5 L x 7.225 mg/L)or **6.19 x$10^{-5}$ g a.i./square foot** [(2.7 g/acre)/43,560 sq ft/acre)].

When compared to the 48-$LD_{50}$ of 19 µg a.i./bee, it would appear that the potential for adverse impact is low for the 85 ppm application rate.

cc: R. S. Jones, D. Greenway, BPPD Subject File
R. S. Jones, F. T. BPPD, CM2, 9th Floor; 703/308-5071: 08/07/2003

*Confidential Statement of Formula may be entitled to confidential treatment*

**Zonix™ Biofungicide (File Symbol 72431-R) - List of Rhamnolipid Biosurfactant products currently on the market for non-pesticidal applications**

| ITEM | APPLICATION PRODUCT | DATE OF MARKET INTRO-DUCTION | BRAND NAME (In some cases biosurfactant is used or sold as Jeneil Biosurfactant Company JBR series product.) |
|---|---|---|---|
| 1 | Crude oil tank cleaner | 3/89 | Petrogen - Ben 22 Biosurfactant<br>Idrabel - RECO215 Biosurfactant |
| 2 | Shampoo | 3/99 | FinnSusp - JBR515 in proprietary formulation. Name not identified by customer |
| 3 | Oil dispersant, oil clean-up, sludge remediation | 10/02 | GFR Biosystems - Bio-Surf<br>Foss Environmental - PES-51 Cleansing Agent<br>Ecochem – EC-601 Oil Slick Dispersant |
| 4 | Fuel additive | 12/02 | Pendragon Holdings - PD-5 Fuel Conditioner |
| 5 | Agricultural adjuvant | 5/00 | Brandt - JBR425 Biosurfactant<br>Mission Organics - JBR425 Biosurfactant |
| 6 | Skin creams, cosmetics | 6/94 | Iwata Chemical Company - Not identified by company |
| 7 | Cleaning products | 7/97- 3/03 | Ecover - BioSquirt<br>BioFuture - Not identified by customer |
| 8 | Soil remediation | 9/00 | Bio-Soil - JBR215 Biosurfactant<br>WRS Infrastructure and Engineering - JBR205 Biosurfactant<br>InterBio - JBR215 Biosurfactant<br>Micro-Bac International -Not identified by customer<br>Bio-Climax - JBR325 and 425 Biosurfactant |
| 9 | Liposome | 6/02 | JBR599 Biosurfactant |
| 10 | Parts cleaner, degreaser | 10/02 | GFR Biosystems Bionetix L-5000, Bionetix L-8500 |

# DATA EVALUATION RECORD

## ZONIX BIOFUNGICIDE

### STUDY TYPE: Honey Bee Acute Contact Toxicity (850.3020)

**MRID 45785201**

Prepared for
Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by
Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37830
Task Order No. 03-05

Primary Reviewer:
Anthony Q. Armstrong, M.S.

Signature: Anthony Q. Armstrong
Date: MAR 25 2003

Secondary Reviewers:
Patricia H. Reno, M.S.

Signature: Patricia H. Reno
Date: MAR 25 2003

Robert H. Ross, M.S., Group Leader

Signature: Robert H. Ross
Date: MAR 25 2003

Quality Assurance:
Lee Ann Wilson, M.A.

Signature: L. A. Wilson
Date: MAR 25 2003

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

## DATA EVALUATION RECORD

**Reviewed by:**

**Secondary Reviewer: Russell S. Jones, Ph.D.**

| | |
|---|---|
| **STUDY TYPE:** | Honey Bee Acute Contact Toxicity (850.3020) |
| **MRID NO:** | 45785201 |
| **DP BARCODE:** | D287603 |
| **CASE NO:** | 070286 |
| **SUBMISSION NO:** | S624790 |
| **TEST MATERIAL:** | Zonix Biofungicide |
| **PROJECT NO:** | 47649 |
| **SPONSOR:** | Jeneil Biosurfactant Company<br>400 N. Dekora Woods Boulevard<br>Saukville, Wisconsin 53080 |
| **TESTING FACILITY:** | ABC Laboratories, 7200 E. ABC Lane, Columbia, MO 65202 |
| **TITLE OF REPORT:** | Acute Contact Toxicity of Zonix (Rhamnolipid Biosurfactant) to the Honeybee, *Apis mellifera* |
| **AUTHORS:** | John Aufderheide, Christopher Hughes |
| **STUDY COMPLETED:** | October 8, 2002 |
| **GOOD LABORATORY PRACTICE:** | GLP Compliant |
| **CONCLUSION:** | A waiver from non-target honeybee testing has been granted for Zonix biosurfactant based on the previously submitted request by the registrant; however, the registrant has recently provided a study report for acute contact toxicity to honeybees. In this study, the effects of Zonix on honeybees via direct contact were assessed at 1.9, 3.2, 5.4, 9.0, 15 and 25 µg a.i./bee in a 48-hour test. Respective mortality was 0, 13, 13, 23, 27 and 77%. No mortality was observed in the untreated control or the carrier control at any time. The 4-, 24- and 48-hour acute contact $LD_{50}$ values for Zonix were >25, 20 and 19 µg a.i./bee. |
| **CLASSIFICATION:** | Acceptable. |

I. **TEST MATERIAL:** Zonix (Rhamnolipid Biosurfactant) Purity: 9.66% rhamnolipids (CAS #s 37134-61-5 and 4348-76-9)

II. **METHODS:** Adult honeybees (*Apis mellifera*) of the same age were obtained from a commercial apiary (Gibbons Honey Farm, Rocheport, Missouri). During holding/acclimation prior to the definitive test, bees were maintained in cages and allowed *ad libitum* access to sucrose/water solution. Test chambers were screened bee cages (14 cm wide x 20 cm long x 10 cm high). Ten bees were impartially placed in each chamber. Testing was initiated by anesthetizing bees in the entire cage by placing them in a $CO_2$ atmosphere and application of a 1 µL drop of dosing solution to the dorsal side of the thorax of each bee. A range finding study was initiated at doses of 0.25, 2.5 and 25 µg a.i./bee in three replicate cages (10 bees/cage). Mortality was 0, 0, 0 and 83 % in the control, 0.25, 2.5 and 25 µg a.i./bee groups, respectively. Based on these results, definitive test concentrations were 1.9, 3.2, 5.4, 9.0, 15 and 25 µg a.i./bee. The untreated control bees were treated with water only and a positive control (dimethoate) was also applied to bees. Each treatment was replicated in three cages for a total of 30 bees per treatment. Bees were incubated for 48 hours in a temperature controlled chamber at 25 ± 2°C and approximately 50 to 70% relative humidity. Mortality and behavioral observations were performed at 4, 14 and 48 hours after treatment had been administered.

III. **RESULTS:** Mortality was 0, 13, 13, 23, 27 and 77% for the bees exposed to treatments of 1.9, 3.2, 5.4, 9.0, 15 and 25 µg a.i./bee, respectively. No mortality was observed in the untreated control or the carrier control at any time (Table 1). In the 3.2 µg a.i./bee and higher treatment groups, abnormal behavior (i.e., lethargy and bees lying on their backs) was observed in some surviving bees. The Zonix 4-, 24- and 48-hour acute contact $LD_{50}$ values were >25, 20 (95% confidence limits of 18 and 22) and 19 (95% confidence limits of 16 and 23) µg a.i./bee.

Mortality in the positive control test ranged from 0 to 100% in the individual dimethoate treatments. Mean mortality after 24 hours exposure was 0, 97 and 100% in bees exposed to 0.020, 0.20 and 0.40 µg dimethoate, respectively. The 24-hour $LD_{50}$ for dimethoate was 0.066 (95% confidence limits of 0.060 to 0.073) µg/bee which is consistent with historical dimethoate values obtained at ABC Laboratories.

IV. **STUDY AUTHOR'S CONCLUSIONS:** Mortality was 0, 13, 13, 23, 27 and 77% for the bees exposed to treatments of 1.9, 3.2, 5.4, 9.0, 15 and 25 µg a.i./bee, respectively. No mortality was observed in the untreated control or the carrier control at ant time. The 4-, 24-and 48-hour acute contact $LD_{50}$ values were >25, 20 and 19 µg a.i./bee for Zonix.

V. **REVIEWER'S CONCLUSION:** The reviewers agree with the study author's conclusions stated above. However, clarification of the relationship between the honeybee 4-, 24- and 48-hour $LD_{50}$ values of >25, 20 and 19 µg a.i./bee and the recommended Zonix field application rate of 85 ppm is needed to more clearly define that the potential for adverse impact is low.

Submitted documents list several application rates ranging from 85 to 100 ppm. The label lists 85 ppm with 8.5% a.i which converts to an applied a.i. rate of 7.225 mg a.i./L or 7.225 µg a.i./mL. When compared to the $LD_{50}$ of 19 µg a.i./bee, it would appear that the potential

for adverse impact is low for the 85 ppm application rate. Furthermore, the honeybee study does not state clearly that a.i. is equivalent to measured rhamnolipids but the reviewers assume that is true.

**BPPD Reviewer Note**: If applied according to label directions at 100 gallons (378.5 L)/acre, the active ingredient is applied at a rate of approximately **2.7 g/acre** (378.5 L x 7.225 mg/L) or **6.19 x10$^{-5}$ g/square foot** [(2.7 g/acre)/43,560 sq ft/acre)] .

**TABLE 1. Mortality and behavioral observations for 48-hour contact exposure of honeybees to Zonix.**

| Nominal Dose (µg a.i./bee) | *4-Hour* | | *24-Hour* | | *48-Hour* | | Cumulative Mean % Mortality |
|---|---|---|---|---|---|---|---|
| | Mortality[1] | Obs.[2] | Mortality[1] | Obs.[2] | Mortality[1] | Obs.[2] | |
| Control | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Carrier Control | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1.9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3.2 | 3.3 | 1 | 3.3 | 1 | 13.3 | 1 | 13 |
| 5.4 | 0 | 6 | 6.6 | 5 | 13.3 | 4 | 13 |
| 9.0 | 6.6 | 8 | 20 | 4 | 23.3 | 4 | 23 |
| 15 | 3.3 | 8 | 13.3 | 5 | 26.6 | 2 | 27 |
| 25 | 36.6 | 15 | 73.3 | 3 | 76.7 | 2 | 77 |

[1]Percent mortality out of 30 bees.

[2]Observations - number of live bees with observed lethargy or lying on their backs. All other alive bees appeared normal.

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| | |
|---|---|
| Chemical: | Decanoic acid, 3-[[6-deoxy-2-o-(6-deoxy-alpha-L-mannopyranosyl)-alpha-L-mannopyranosyl]oxy]-, 1-(carboxymethyl)octyl ester, mixt. with 1-(carboxymethyl)octyl 3-[(6-deoxy-alpha-L-mannopyranosyl)oxy]decanoate |
| | PC Code: 110029 |
| HED File Code: | 41300 BPPD Eco Effects |
| Memo Date: | 8/7/2003 |
| File ID: | DPD289391<br>DPD289392 |
| Accession #: | 000-00-9002 |

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